Gene therapy

THE MUCH HERALDED REVOLUTION IN GENE THERAPY, PROMISED AT THE BEGINNING OF THE 1980S, IS GRADUALLY MAKING ITS WAY TO THE PATIENT, BUT, AS MOST GENE THERAPISTS WOULD ADMIT, PROGRESS IS FAR SLOWER THAN ONCE PREDICTED. CLARE THOMPSON ASKS RICHARD A MORGAN (NIH) AND R MICHAEL BLAESE (KIMERAGEN, PENNSYLVANIA) ABOUT THE HURDLES THAT NEED TO BE JUMPED BEFORE THE FULL POTENTIAL OF THE TECHNOLOGY CAN BE REALISED.

THE IMPACT OF NEW TECHNOLOGIES IN MEDICINE
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GENE THERAPY FOR GENETIC DISORDERS

Blaese has had first hand experience of these problems. Ever since he administered the first ever gene therapy to Ashanti De Silva, a 4 year old girl suffering from the inherited immunodeficiency adenine deaminase deficiency, he has been at the forefront of research and development into the technology.

The case began in September 1990. Ashanti had been living with her genetic disease for four years and had many of the typical features of immunodeficiency, such as recurrent infections and poor growth. Her clinicians were keeping the disease at bay with a new enzyme replacement therapy—polyethylene glycol adenine deaminase (Peg ADA)—the cost of which amounted to between $350 000 and $400 000 a year.

Blaese's team would have liked to have inserted the gene for adenine deaminase into one of Ashanti's stems cells, to enable them to produce a continuous stream of fully functioning T lymphocytes. Unfortunately, however, says Blaese, "The technology just wasn't there."

Instead, the team took some of Ashanti's peripheral blood, separated the T cells by apheresis, and then grew them in culture, using the cytokine interleukin 2 to stimulate growth and the antibody OKT3 to stimulate the T cell receptors to push the cells to divide. They then added the gene for adenine deaminase to the cells via a retrovirus vector.

"The T cells grew over 10 days," says Blaese. "We reinfused them back into Ashanti over a year. We saw the peripheral T cell count increase from 1000 to over 3000, and her ADA levels increased to about a quarter of normal." The team also saw recovery of her immune system: "She made an antibody response to tetanus toxoid and haemophilus B vaccine, and we can still see an awakened immune response in both her T and B cells nine years later."

But, as with many gene therapy experiments, many commentators have said that the results are open to interpretation. The biggest confounding factor was that Ashanti had to continue taking the enzyme replacement therapy as a back up. Blaese, however, rejects this criticism, pointing to the recovery of her immune system as proof of concept: "She was on PEG ADA enzyme replacement for two years before the experiment, and she had a lousy immune system then."

Today, Ashanti is still well, plays soccer, and takes no extraordinary precautions against immunodeficiency. The team, however, are still modifying their strategy: "We have developed a new ADA vector which uses a different virus package and is 50 times more powerful than the original. We are going to try and insert it into a stem cell, and the NIH will treat her in the fall of this year."

GENE THERAPY FOR CANCER

As opposed to the treatment Ashanti received, in which the aim was simply to introduce a gene that is missing, some gene therapy approaches are intended to enhance existing clinical therapies. One of the experimental approaches to the treatment of localised cancers, including brain and liver tumours, involves in vivo delivery to cancer cells of genes encoding viral or bacterial enzymes involved in the conversion of non-toxic product to their active molecules. One of these approaches involves taking the thymidine kinase gene from the herpes simplex virus and transferring the gene into tumour cells. This gene codes for a viral enzyme that makes the cells much more susceptible to the antiviral drug ganciclovir.

In one particular trial 15 patients with advanced recurrent brain tumours were treated with cells carrying a retrovirus containing the thymidine kinase gene. The virus vector was allowed to spread in the tumour for a few days, and then all the patients received two weeks of ganciclovir treatment.

Overall, the trial was not a success, but one patient, who had three lesions, astounded all the expectations: "Over a period of six years all three of his lesions regressed, and there has been no recurrence." But, as Blaese readily admits, this is not the whole answer because, of the other 14 patients, only one other showed any regression. "Certain individuals can respond. In many gene therapy trials you have to learn as much as you can from the patient and then go back to the lab."

GENE THERAPY FOR AIDS

In theory, many of the new gene therapy protocols should work. It's just the details that seem to get in the way. An example of this problem is illustrated by a trial of gene therapy for HIV infection that is currently taking place at the City of Hope National Medical Center in Duarte, California. The team, led by Dr John Zaia, hit on an ingenious scheme to keep HIV at bay. The idea was based on the premise of fighting the virus from within, by inserting a chemical into cells that would stop the virus from replicating.

The treatment consisted of taking stem cells from people infected with HIV and transducing them in the laboratory with a retroviral vector called the Moloney virus. The virus also contained ribozymes that would block the expression of key HIV proteins called TAT and REV. The idea was that, if the expression of these proteins was blocked, the HIV would be unable to replicate. Once the genetically modified stem cells were reinfused into the patients, they would give rise to HIV-proof immune cells.

In theory it sounded fine, and in the laboratory the team managed to get the viral construct into 30-50% of the stem cells that they transduced. Then they decided to test the procedure in HIV positive people. "In the first phase of the study we treated five healthy HIV positive people," says Zaia. "They hadn't developed AIDS." The treatment seemed to be safe, but the problem came with the expression of the gene. "Only three people showed evidence of genetic marking," says Zaia, "and that was between three and six months after infusion. We never saw very high marking—only about one in a million cells."

The team obviously had a problem getting the cells to engraft. "We argued that you might have to eradicate the bone marrow so that the genetically modified stem cells can find space in which to engraft properly," says Zaia. The team decided on another tack. It would be unethical to remove bone marrow from healthy patients with HIV infection, but patients with AIDS and high grade non-Hodgkin's lymphoma could conceivably benefit from bone marrow eradication with high dose chemotherapy. So, for each of four such patients, the team reinfused the patient's own genetically modified stem cells after high dose chemotherapy.

"The first patient died after 115 days of a relapsed lymphoma but had high levels of gene expression—between 1 in 10 000 and 1 in 50 000 at the time of his death. The three other patients are alive and well, but only one is past the three month mark, so it is too early to know if continued gene expression will occur," says Zaia.

The problem of delivering the gene to the right place is one that seems to haunt many gene therapy experiments. "If we could get a good vector into the system, then we might just win," says Zaia. "That sets the stage for using a lentivirus or an adeno-associated virus."

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